

Tsang et al. Bone 2019 - Supplemental figures

Fig. S1. Loss of *Bmal1* in osteoclasts does not result in reduced bone mass. Femurs from 8-week-old male *Bmal1^{ff/+}.Ctsk-cre* and *Bmal1^{ff/ff}.Ctsk-cre* littermates were analyzed by microCT. (A) Trabecular bone parameters, (B) trabecular BMD, (C) cortical thickness, and (D) cortical BMD. Bars are mean and SD; n=9 animals per group; p values by Student's *t* test.

Fig. S2. *Cathepsin K-cre* knock in mice do not have a bone phenotype at 8 weeks of age. Femurs from 8-week-old male C57BL/6 WT and *Ctsk-cre* littermates were analyzed by microCT. (A) Trabecular bone parameters, (B) trabecular BMD, (C) cortical thickness, and (D) cortical BMD. Bars are mean and SD; n=8-9 animals per group; p values by Student's *t* test.

Fig. S3. *Bmal1* heterozygosity does not result in reduced bone mass. Femurs from 8-week-old male *Bmal1^{+/+}* and *Bmal1^{+/-}* littermates were analyzed by microCT. (A) Trabecular bone parameters, (B) trabecular BMD, (C) cortical thickness, and (D) cortical BMD. Bars are mean and SD; n=11 animals per group; p values by Student's *t* test.

Fig. S4. Real-time quantitative PCR assay to measure *Bmal1* gene deletion in *Bmal1^{ff}.cre* mice. (A) Schematic representation of the floxed *Bmal1* locus (*Bmal1^{fllox}*) where exon 8 is flanked by *loxP* sites ⁽⁷⁾. Primer pair FL5 surrounds the proximal *loxP* site. *D7Mit97* is a nearby microsatellite marker. Upon cre-mediated recombination, exon 8 is excised resulting in the *Bmal1^Δ* allele and loss of the FL5 PCR product. (B) Germline DNA from genotyped *Bmal1^{+/+}*, *Bmal1^{+/-}*, *Bmal1^{-/-}*, *Bmal1^{ff/-}* and *Bmal1^{ff/+}* mice was amplified using the *D7Mit97* and FL5 primer pairs. PCR products were separated on a 2.5% Agarose gel. Note that the *Bmal1^{fllox}* allele is

longer than the wild-type *Bmal1*⁺ allele, whereas no PCR product is detected with DNA from the *Bmal1*^{-/-} mouse.

Fig. S5. Female *Bmal1*^{ff}.*Prx-cre* mice have reduced bone mass. Femurs from 8-week-old female *Bmal1*^{ff} and *Bmal1*^{ff}.*Prx1-cre* littermates were analyzed by microCT. (A) Trabecular bone parameters, (B) trabecular BMD, (C) cortical thickness, and (D) cortical BMD. Bars are mean and SD; n=10 animals per group; p values by Student's *t* test.

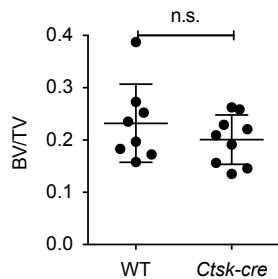
Fig. S6. *Bmal1* deletion and disruption of the circadian molecular clock in *Bmal1*^{ff}.*Prx-cre* mice. (A) Expression of *Bmal1* and the clock genes *Clock*, *Period1*, *Period2* and *Nr1d1* was measured by quantitative real-time PCR in cortical bone of 8-week-old male *Bmal1*^{ff} and *Bmal1*^{ff}.*Prx1-cre* mice. Each data point represents one animal; bars are mean and SD; p values by Student's *t* test. Specimens were collected at circadian time 6 hours. (B) To illustrate that analysis at a single time point does not capture the dynamic changes of clock gene expression over a 24-hour period, the expression of molecular clock genes in the Achilles tendon of 4-week-old male *Bmal1*^{ff} and *Bmal1*^{ff}.*Prx1-cre* mice is shown. This experiment was performed as part of an unrelated study. Specimens were collected every 6 hours. The X-axis represents circadian time. Data are mean and SD (n=3 mice per genotype and time point). Arrows highlight the timepoint (i.e. circadian time 6 hours) when bones were harvested for the experiment in (A).

Fig. S7. Osteoclast differentiation *in vitro* is not altered in *Bmal1*^{ff}.*Prx1-cre* mice. (A) TRAP staining of representative osteoclasts differentiated from *Bmal1*^{ff} and *Bmal1*^{ff}.*Prx1-cre* bone marrow in the presence of M-CSF and RANKL. (B) Quantitation of formation of TRAP+ multinucleated osteoclasts from *Bmal1*^{ff} and *Bmal1*^{ff}.*Prx1-cre* bone marrow. (C) Enzymatic

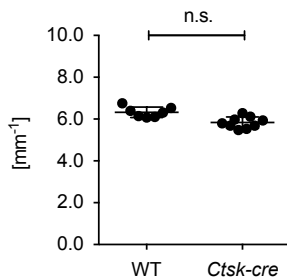
activity of secreted TRAP measured by colorimetric assay on the supernatants of *Bmal1^{ff}* and *Bmal1^{ff}.Prx1-cre* bone marrow cells cultured in the presence of M-CSF alone or M-CSF and RANKL. Each symbol represents one animal.

A

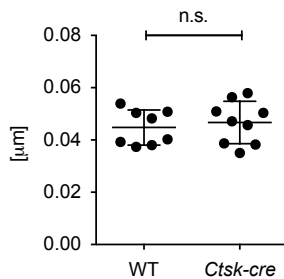
Tb BV/TV



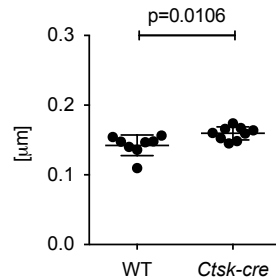
Tb N



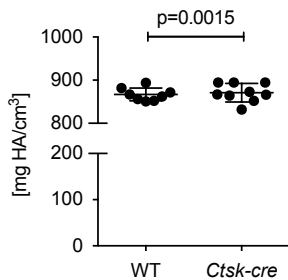
Tb Th



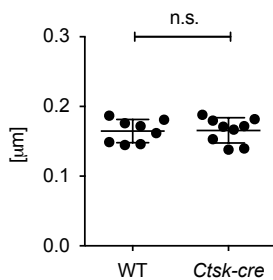
Tb S

**B**

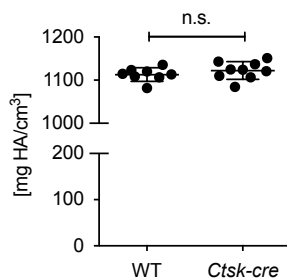
Tb BMD

**C**

Cort Th

**D**

Cort BMD

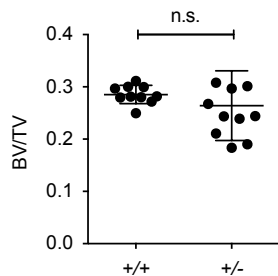


C57BL/6 (WT) vs.
Ctsk-cre
male, 8 weeks

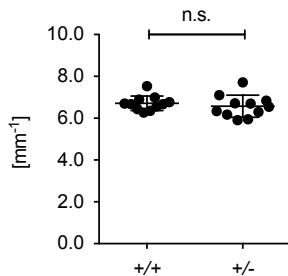
Figure S2

A

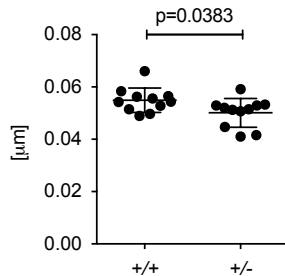
Tb BV/TV



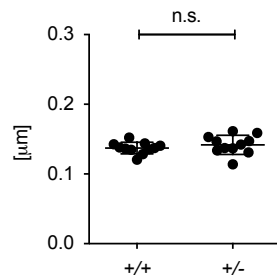
Tb N



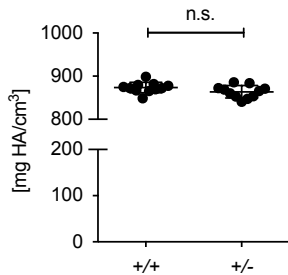
Tb Th



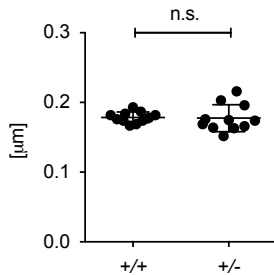
Tb S

**B**

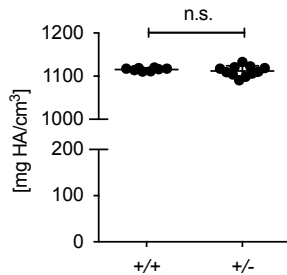
Tb BMD

**C**

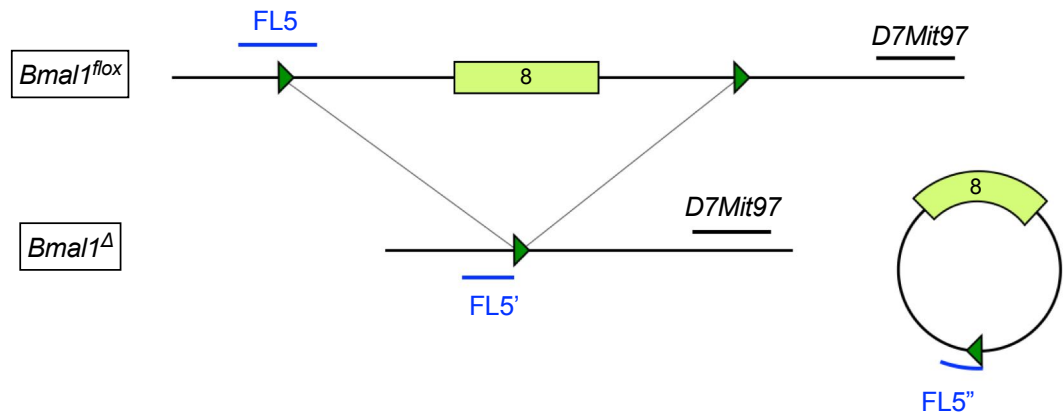
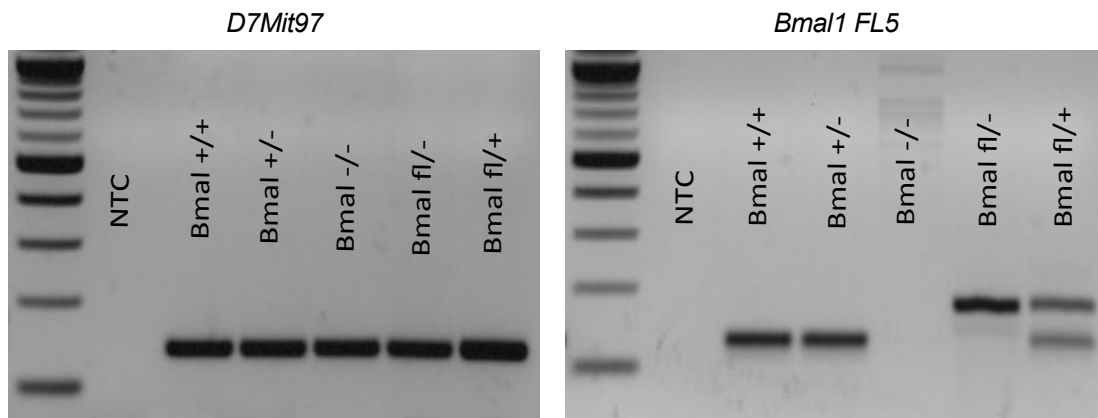
Cort Th

**D**

Cort BMD



Bmal1^{+/+} vs. *Bmal1*^{+/-}
male, 8 weeks

A**B****Figure S4**

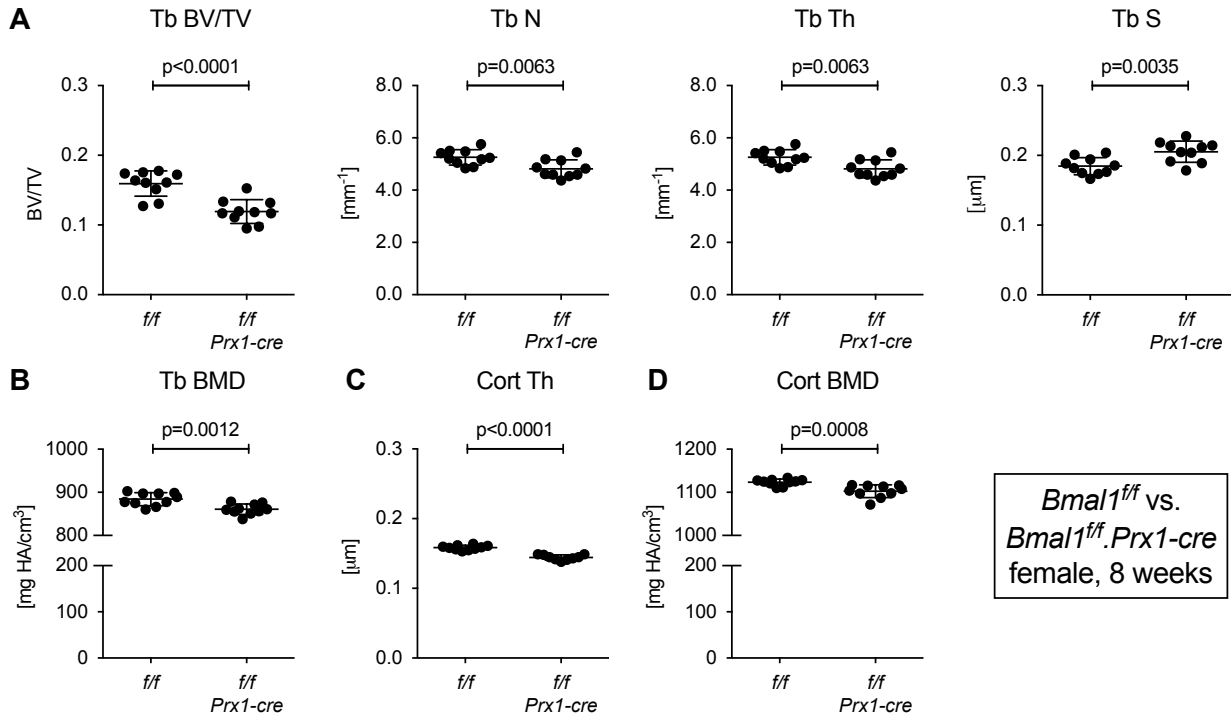
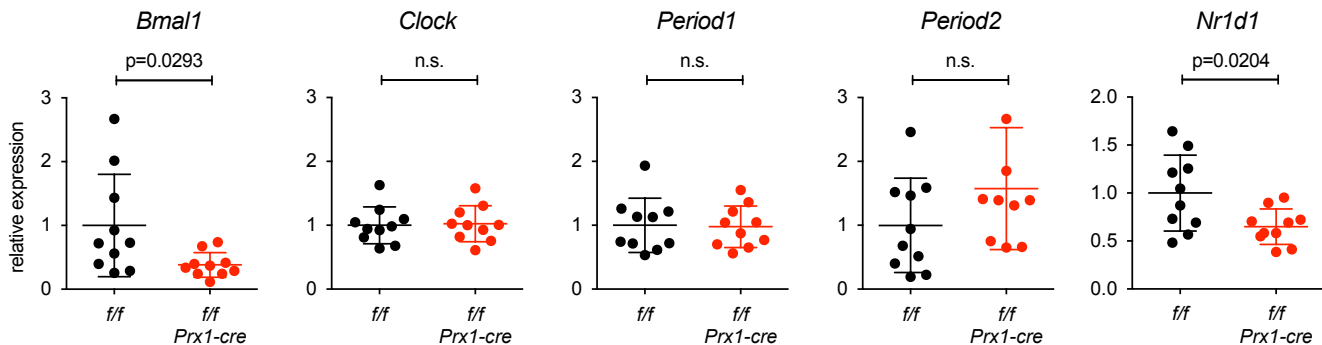


Figure S5

A Cortical bone at circadian time 6 hours



B Achilles tendon

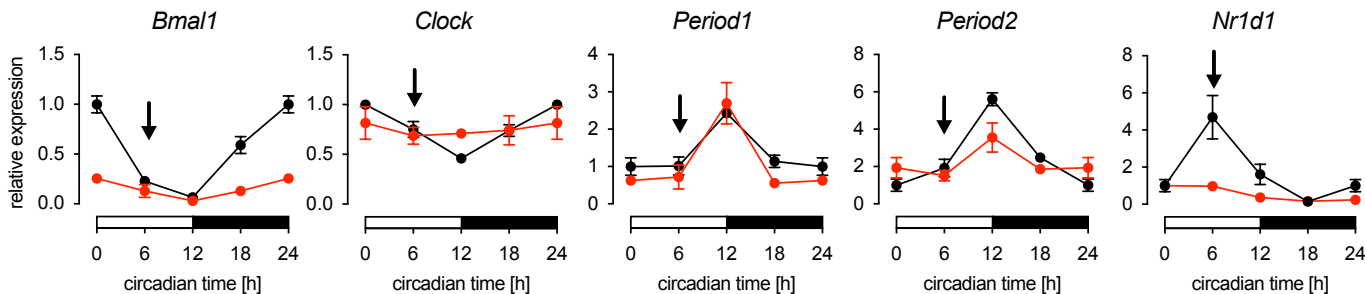
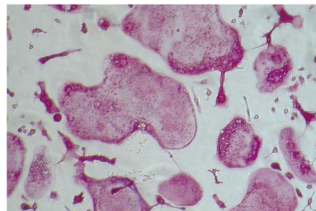
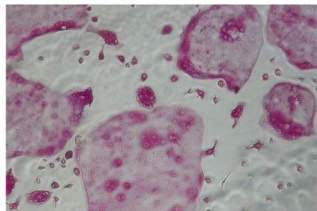
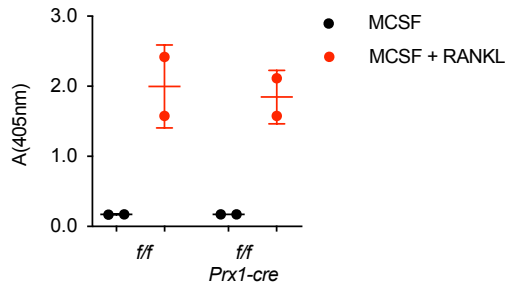
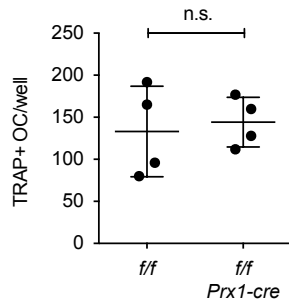


Figure S6

A*Bmal1^{ff}**Bmal1^{ff}.Prx1-cre***B****Figure S7**